Effect of Relative Humidity and Temperature on Airborne Venezuelan Equine Encephalitis Virus

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Inactivation of airborne Venezuelan equine encephalitis (VEE) virus disseminated from liquid suspensions or from lyophilized preparations as 1- to 5-μm particles was investigated under various conditions of relative humidity and temperature in a 2,500-liter static aerosol chamber. Relative humidity ranging from 18 to 90% at 24 C and temperature ranging from −40 to 24 C had no marked effect on the biological decay rate or the recovery of viable airborne VEE virus disseminated from liquid suspensions. However, at 49 C a significant increase in the biological decay rate and decrease in aerosol recovery of the VEE virus were observed. Airborne lyophilized VEE virus was significantly affected by relative humidity. An increase in relative humidity from 20 to 90% resulted in progressive decrease in aerosol recovery of viable VEE virus. A twofold reduction in aerosol recovery of the lyophilized virus was observed at and above 29 C as compared to the lower temperatures studied. However, the differences among biological decay rates of lyophilized VEE virus were not significant within temperature range of −40 to 38 C.

Infection by the respiratory route represents the primary hazard to personnel engaged in studies of pathogenic microorganisms. Furthermore, due to potential cross-contamination, airborne spread of infection can significantly influence the results of experimental studies involving laboratory animals. Because of the magnitude of this problem, considerable amount of information has been compiled in recent years concerning the relationship between the performance of conventional microbiological laboratory procedures and the presence of airborne microorganisms. The available evidence suggests that common laboratory manipulations can result in a release of microorganisms which, if pathogenic, can serve as sources of infection. The largest group of agents implicated in laboratory acquired infections are the arboviruses and, most predominantly, the Venezuelan equine encephalitis (VEE) virus (12, 13).

Once airborne, microorganisms are exposed to a variety of environmental stresses, the most important ones being effects of temperature and relative humidity (RH). Because of the high potential hazard, the use of infectious viruses in aerosol studies presents many problems. Thus, frequently the aerosol stability of infectious viruses is extrapolated from data obtained in ex-

periments with noninfectious viruses, such as coliphage. This study was conducted to provide direct data on the survival of airborne VEE virus under various conditions of temperature and RH. The aerosol parameters of interest were aerosol recovery and biological decay rate of VEE virus disseminated from liquid suspensions or lyophilized preparations.

MATERIALS AND METHODS

Virus. A frozen chick embryo suspension, a lyophilized preparation of the Trinidad strain of VEE virus, and a lyophilized or a frozen concentrated slurry of spores of *Bacillus subtilis* var. *niger* (Batch no. 91) were kindly provided by the U.S. Army, Fort Detrick. The microorganisms were stored in vials at dry-ice temperature until used.

To study the effects of RH, frozen VEE virus was thawed at 24 C, briefly blended in a Waring Blendor, screened through a 100-mesh stainless-steel screen, distributed into 2.0-ml portions, and stored at dry-ice temperature until used.

For studies of the effects of temperature, spores of *B. subtilis* were used as the physical decay tracer. The frozen VEE virus was thawed at 24 C, and 2.0 g of frozen *B. subtilis* spores was added to 50 ml of the virus suspension. The mixed suspension was briefly blended, screened, distributed into 3.0-ml samples, and stored at dry-ice temperature until used.

For dissemination, 1.0 ml of the liquid VEE virus-

B. subtilis spores mixture or the pure VEE suspension was used in a two-fluid atmoizer (FK-8 aerosol gun). The lyophilized VEE virus and VEE virus-*B. subtilis* mixtures were disseminated by means of an air-blast nozzle, designated the OL tube (8). Both atomizers produced aerosol clouds having the majority of particles in the 1- to 5-μm size range, regardless of the agents used for the dissemination.

Aerosol chamber. The VEE virus preparations were disseminated into a 2,500-liter, Freon-tight, stainless-steel aerosol chamber with an attached series of microbiological safety cabinets (class III hoods). To maintain a uniform distribution of the aerosol, a small fan was operated in the chamber throughout the experimental trials. The Freon-tight cabinets provided isolated space for dissemination of the virus preparations and for sampling the aerosol and animal exposures, as well as suitable areas for the inoculation and incubation of tissue cultures. Materials were passed in and out of the cabinet system through a double door, interlocking autoclave, and germicidal bath tank arrangement. All work in the cabinets was done through attached arm-length heavy rubber gloves. The aerosol chamber and the associated cabinet system were maintained under negative pressure of 0.5 to 1.0 inches of water. The exhaust air was incinerated, and the liquid effluents were sterilized before disposal.

Temperature and RH in the aerosol chamber were established at the desired level before dissemination of the virus preparations. The temperature and humidity were maintained and continuously monitored throughout all aerosol trials (4). Air temperature in the chamber was measured and recorded with a resistance bulb connected with a Foxboro model 694 resistance-to-current converter. The dew point was determined by means of a Foxboro model 2711 AG electronic-type humidity-sensitive dew cell element. The dew point measurements were converted to per cent RH by using standard conversion tables.

During the studies of the effects of RH on inactivation of airborne VEE virus, the humidity in the aerosol chamber ranged from 18 to 90 $\pm$ 5% RH and the temperature was maintained at 24 $\pm$ 2 C. To study the effects of temperature on the viral aerosol, temperature in the chamber ranged from -40 to 49 $\pm$ 2 C. At -2 C and above the humidity was maintained at 70 $\pm$ 5% RH, whereas below this temperature ambient RH was used.

Aerosol sampling and assay. The aerosol in the chamber was sampled for 1 min at various cloud ages with two parallel all-glass impingers (AGI-30) operated at a sampling rate of 12.5 liters per min (1). A single-stage impactor (10) designed to remove particles larger than 5 μm was used, instead of the conventional curved stem of the AGI-30. The impingers contained 20 ml of sampling fluid consisting of Heart Infusion broth with 0.15% Dow-Corning antifoam A emulsion. To prevent freezing of the sampling fluid during experiments involving subfreezing temperatures, the AGI-30 samplers were positioned in a water bath maintained at approximately 20 C.

The contents of the duplicate samplers were pooled for quantitative assay of the airborne microorganisms. The viable VEE virus was assayed by the plaque technique in primary chick monolayer tissue culture. The plates were incubated at 37 $\pm$ 1 C for 72 hr, and the plaques were counted. *B. subtilis* was assayed conventionally on Tryptose agar (3).

Decay of the VEE virus aerosol was estimated by quantitating the numbers of viral plaque-forming units (PFU) airborne in particles of 1 to 5 μm diameter per unit of air volume at various cloud ages. The slope of the resulting curve described the rate of aerosol decay and was expressed as per cent per minute. This total decay rate included losses due to physical factors, based on aerosol recovery of spores of *B. subtilis*, and those due to virus inactivation. Estimates of the physical decay of the aerosol were determined on the basis of the recovery of spores of *B. subtilis* disseminated as an intimate mixture with the VEE virus (3). The difference between the total decay rate and the physical decay rate provided an estimate of the death rate of the airborne VEE virus.

The reported mean biological decay rates (death rates) and aerosol recoveries were based on results from a minimum of six replicate aerosol trials conducted at each experimental condition. The means were compared by standard analysis of variance technique. The significance of the differences is reported at the 5% probability level.

RESULTS

Relative humidity. In studying the effects of relative humidity on the aerosol behavior of VEE virus, spores of *B. subtilis* were not used to determine the physical decay rates of the cloud. However, numerous aerosol experiments conducted with wet and dry preparations of the VEE virus and other microorganisms containing a physical tracer in the 1- to 5-μm particle size indicated a physical decay rate in the aerosol chamber of approximately 1.5% per min (3, 4). Thus, for these experiments this value was used to arrive at the estimate of the death rate of the airborne VEE virus.

Figure 1 shows actual and mean aerosol recovery of viable VEE virus at various cloud ages disseminated from liquid suspensions at 24 C and humidity ranging from 18 to 90% RH. At 4 min of cloud age, the aerosol recovery of the virus ranged from 7.1% at 78% RH to 13.8% at 18% RH. Although the observed differences were significant, there was no orderly relationship between RH and aerosol recovery of VEE virus. The differences among the death rates of this liquid VEE virus preparation at various RH levels were not significant. The biological decay rates of the airborne VEE virus ranged from 0.9 to 2.0% per min, with a mean death rate of 1.3% per min calculated across the seven RH conditions.
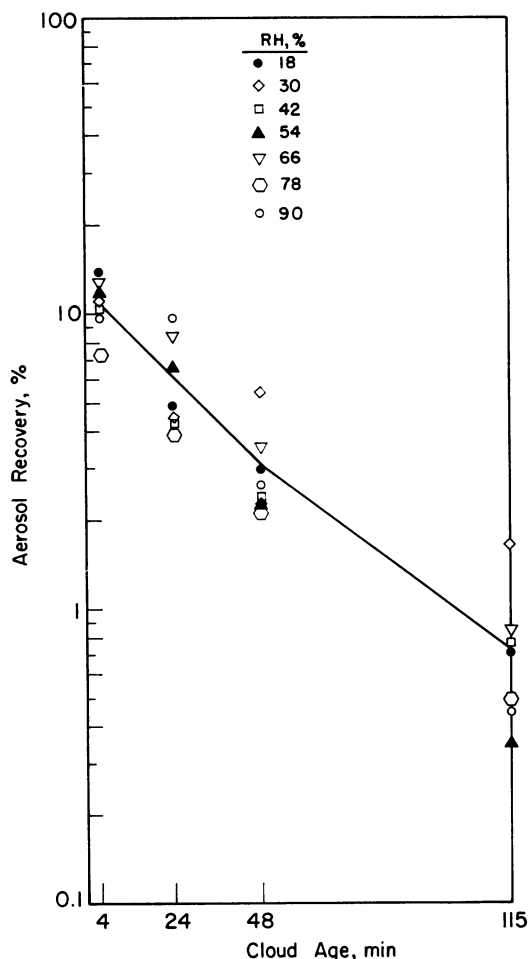
FIG. 1. *Effect of relative humidity at 24 C on aerosol recovery of VEE virus disseminated from liquid suspensions.*

Figure 2 summarizes the aerosol recovery of VEE virus disseminated as a lyophilized preparation in 1- to 5-μm particle size range. The data suggest a close relationship between aerosol recovery of the viable virus and RH. A progressive increase in humidity from 20 to 62% RH resulted in an almost linear decrease in recovery of the airborne VEE virus from aerosol clouds studied over a 60-min time period. At 76 and 90% RH, an approximately one-log decrease in aerosol recovery was observed as compared to the lower humidities. The data suggest that the effects of humidity on aerosol recovery of the lyophilized VEE virus could be divided into four significant levels of relative humidity, namely (i) 20%, (ii) 34 to 48%, (iii) 62%, and (iv) 76 to 90% RH; each increment of humidity resulted in decreased recoveries of viable VEE virus.

The effects of humidity on the biological decay rate of lyophilized airborne VEE virus can be grouped in two ranges. The mean biological decay rate of VEE virus calculated across the four points within 20 to 62% RH was 2.8% per min and ranged from 2.3 to 3.2% per min. The mean biological decay rate for the second group of humidities (76 to 90% RH) was 5.7% per min and ranged from 5.3 to 6.1% per min. The increased mean biological decay rate observed at the high RH was significant as compared to the mean decay within the lower humidity range.

Temperature. In all experiments in which the effects of temperature on airborne VEE virus were investigated, spores of *B. subtilis* were used as physical decay tracer. Thus the reported values reflect the inactivation of VEE virus at various conditions of temperature. Aerosol decay rates of the VEE virus in particle size range of 1 to 5 μm disseminated from liquid or lyophilized preparations are summarized in Table 1.
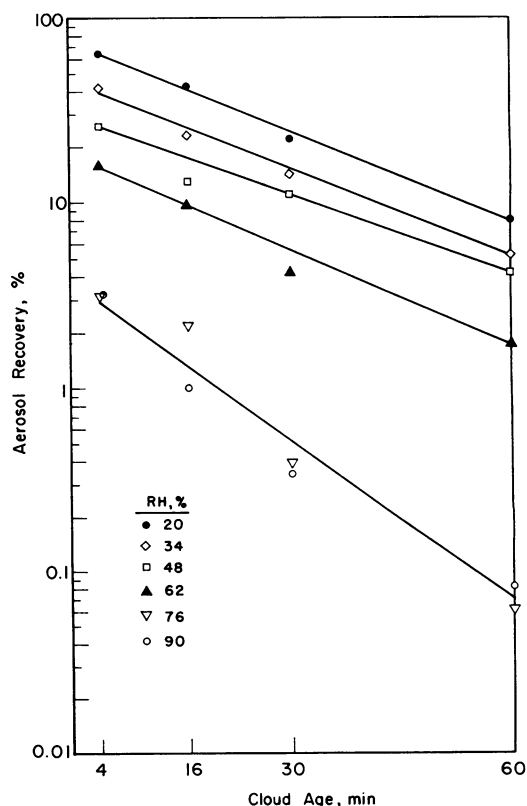
The data indicated that the biological decay

FIG. 2. *Effect of relative humidity at 24 C on aerosol recovery of lyophilized VEE virus.*

TABLE 1. *Effect of temperature on aerosol decay rate of Venezuelan equine encephalitis (VEE) virus*

Aerosol chamber conditions		Death rate (per cent/min)	
Temp (C)	Relative humidity (%)	Liquid VEE virus	Lyophilized VEE virus
−40	Ambient	−0.34	2.24
−18	Ambient	0.58	1.73
−2	70	0.05	2.25
24	70	0.90	2.85
29	70	ND[a]	1.83
38	70	ND	1.57
49	70	3.89	ND
49	90	5.54	ND

[a] ND = not determined.

of VEE virus disseminated from liquid suspensions was significantly higher at 49 C than at the four lower temperatures. The mean biological decay rate within the temperature range of −40 to 24 C was 0.3% per min, whereas at 49 C the decay rate was 4.7% per min. Furthermore, at 49 C the biological decay rate appeared to be higher when the humidity was maintained at 90% than at 70% RH.

Aerosol recovery of VEE virus at various cloud ages (Fig. 3) further illustrates the effect of temperature on airborne VEE virus. A relationship similar to that of the biological decay rate was apparent between temperature and aerosol recovery of the virus. The differences among aerosol recovery values were of no practical importance within temperatures ranging from −40 to 24 C. However, at 49 C the aerosol recovery of VEE virus decreased significantly.

Differences among biological decay rates of the airborne lyophilized VEE virus observed within a temperature range of −40 to 38 C were not significant. The mean biological decay rate calculated across the six temperature points was 2.1% per min (Table 1). Increase of temperature from −40 to 24 C had no effect on the aerosol recovery of the lyophilized VEE virus. However, an increase from 24 to 38 C resulted in an approximately twofold decrease in the aerosol recovery, especially at the early cloud ages. This effect of temperature was confirmed when the per cent aerosol survival of lyophilized VEE virus was calculated on the basis of ratio of the virus to *B. subtilis* initially present in the dissemination mixture, representing 100% recovery, and in the aerosol samples collected at various cloud ages. The per cent survival shown in Fig. 4 suggested that the adverse effect of temperature on lyophilized VEE virus was most
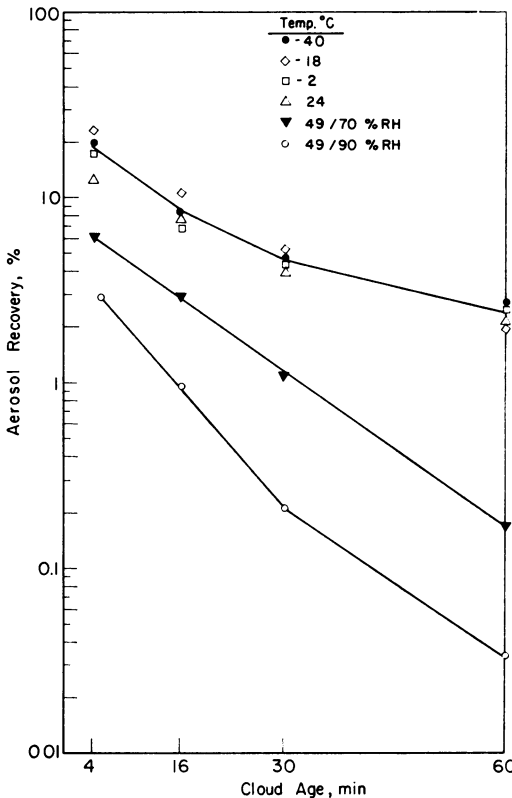

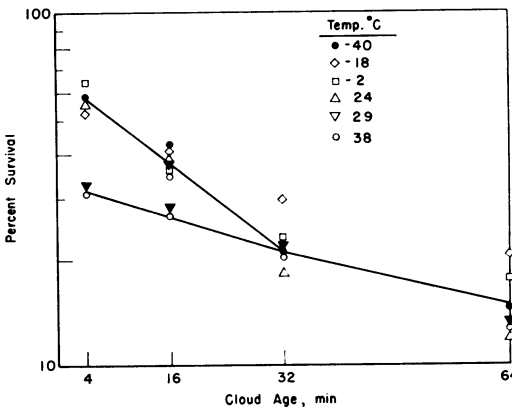FIG. 3. *Effect of temperature on aerosol recovery of VEE virus disseminated from liquid suspensions.*

Figure 4

FIG. 4. *Effect of temperature on aerosol survival of lyophilized VEE virus.*

apparent during the early cloud ages, namely at 4- and 16-min after dissemination. Thereafter, the surviving fraction of the virus did not appear to be affected by the temperature.

DISCUSSION

Although under natural conditions VEE virus is transmitted by the mosquito, ample evidence is available in the literature suggesting that the disease can also be initiated by inhalation of airborne virus particles (2, 7, 9, 11). Thus the effects of environmental humidity and temperature on the survival of VEE virus aerosol can be of major importance to the understanding of the transmission of the disease. This is of special significance when the airborne viral particles are in the 1- to 5-μm size range inasmuch as such particles are most efficiently retained in the respiratory tract.

The experimental data indicate that RH did not have any marked effect on the aerosol characteristics of VEE virus disseminated from liquid suspensions. The aerosol parameters of prime interest, namely the biological decay rate and aerosol recovery, did not differ significantly within a humidity range of 18 to 90% RH at 24 C. The differences among biological decay rates and aerosol recoveries of VEE virus also were not significant within a temperature range of −40 to 24 C. However, at 49 C a more than 10-fold increase in biological decay and a significant reduction in aerosol recovery of the viable airborne virus were observed. Furthermore, at the latter temperature, increase in humidity from 70 to 90% RH appeared to produce a more detrimental effect on the airborne VEE virus.

Harper (5, 6) reported that VEE virus disseminated from liquid suspensions survived best at lower humidities (17 to 25% RH) and at the lowest temperature (9 C) tested. Furthermore, at each level of humidity, namely, 20, 50, and 80% RH, the survival was best at the lowest temperature. However, as pointed out by the author the conclusions were based on a limited number of replicate experiments, thus resulting in a large experimental error. In our studies the experimental error, although still present, was considerably reduced by the performance of six replicate experiments at each environmental condition. Thus, on the basis of the data obtained, it appears that airborne VEE virus is stable and indeed not markedly affected by humidities and temperatures expected in conventional laboratory environments.

Airborne lyophilized VEE virus was much more sensitive to humidity than the virus disseminated from liquid suspensions. A progressive increase in humidity from 20 to 90% RH resulted in progressive decreases of aerosol recovery of the viable VEE virus. The biological decay was significantly higher at 76% RH than at lower humidities. Although the death rate of the

lyophilized virus did not appear to be affected significantly by temperature ranging from −40 to 38 C, at temperatures above 29 C the aerosol recovery of the VEE virus at the early cloud ages was reduced approximately twofold.

A direct comparison of the survival of airborne VEE virus disseminated as liquid and lyophilized preparations was not possible. The data suggest that the initial, 4-min aerosol recovery of the dry VEE virus was generally higher than that of the liquid virus. However, the death rates of the airborne lyophilized virus were markedly higher than those of the liquid VEE virus. Whereas most of the experimental conditions, such as particle size, aerosol sampling rate, collecting fluids, and assay media, were held constant throughout the studies, other factors, such as the efficiency of the disseminating devices and the method of treatment of the virus before the dissemination, varied.

To define the effect of disseminating devices on airborne microorganisms, the aerosol-source strength parameter was estimated for the virus and the highly stable B. subtilis spores. This parameter reflected an extrapolation of the per cent recovery regression to the zero-time cloud age and provided a measure of the efficiency of the disseminating devices. In general, under similar temperature and RH conditions and approximately equal concentration of the microorganisms in the initial materials, the estimates of aerosol-source strength were higher by a factor of two for the dry than for the liquid B. subtilis preparation. A similar ratio was observed when the aerosol-source strength was calculated for the VEE virus. This suggested that under the experimental conditions the device used for the dissemination of the dry material was more efficient and possibly less stressful to the microorganisms. The effects of methods of growth, conditions of lyophilization, reconstitution of the virus with water, and presence of any additives on aerosol survival were not investigated and indeed should be explored.

The evidence presented indicates that, in general, VEE virus in particle size range of 1 to 5 μm is highly stable in aerosol form. Thus, upon escape due to laboratory accident or due to inadequate containment during laboratory operations the VEE virus could persist for extended periods of time and under proper conditions it could become a source of airborne infection.

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